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Medicines from the Deep Sea: Exploration of the Gulf of Mexico

What Killed the Seeds?

Focus
Bioassays

Grade Level
7-8 (Life Science)

Focus Question
How can the biological effects of chemicals be studied?

Learning Objectives
Students will be able to explain and carry out a simple process for studying the biological effects of chemicals.

Students will be able to infer why organisms such as sessile marine invertebrates appear to be promising sources of new drugs.

Materials
- ❒ Radish seeds; at least 20 for each student group
- ❒ 10% household bleach solution
- ❒ Disposable plastic petri dishes, 100 mm x 10 mm (Carolina Biological Supply No. WW-74-1248); at least six for each student group
- ❒ Filter paper cut into 9 cm circles; at least six for each student group
- ❒ Disposable plastic pipettes with rubber bulb or aspirator, one for each student group
- ❒ Distilled water
- ❒ Marker board, blackboard, or overhead projector with transparencies for group discussions

Audio/Visual Materials
None

Teaching Time
One or two 45-minute class periods

Seating Arrangement
Groups of 2-3 students

Maximum Number of Students
30

Key Words
Cardiovascular disease
Cancer
Arthritis
Natural products
Bioassay

Background Information
Despite the many advances of modern medicine, disease is still the leading cause of death in the United States. Cardiovascular disease and cancer together account for more than 1.5 million deaths annually (40% and 25% of all deaths, respectively). In addition, one in six Americans have some form of arthritis, and hospitalized patients are increasingly threatened by infections that are resistant to conventional antibiotics. The cost of these diseases is staggering: $285 billion per year for cardiovascular disease; $107 billion per year for cancer; $65 billion per year for arthritis. Death rates, costs of treatment and lost productivity, and emergence of drug-resistant diseases all point to the need for new and more effective treatments.

Most drugs in use today come from nature. Aspirin, for example, was first isolated from the willow tree. Morphine is extracted from the opium poppy. Penicillin was discovered from common bread mold. To date, almost all of the drugs derived from natural sources come from terrestrial organisms. But recently, systematic searches for new drugs have shown that marine invertebrates produce more antibiotic, anti-cancer, and anti-inflammatory substances than any group of terrestrial organisms. Particularly promising invertebrate groups include sponges, tunicates, ascidians, bryozoans, octocorals, and some molluscs, annelids, and echinoderms.

The list of drugs derived from marine invertebrates includes:

Ecteinascidin – Extracted from tunicates; being tested in humans for treatment of breast and ovarian cancers and other solid tumors

Topsentin – Extracted from the sponges *Topsentia genitrix*, *Hexadella* sp., and *Spongosorites* sp.; anti-inflammatory agent

Lasonolide – Extracted from the sponge *Forcepia* sp.; anti-tumor agent

Discodermalide – Extracted from deep-sea sponges belonging to the genus *Discodermia*; anti-tumor agent

Bryostatin – Extracted from the bryozoan *Bugula neritina*; potential treatment for leukemia and melanoma

Pseudopterosins – Extracted from the octocoral (sea whip) *Pseudopterogorgia elisabethae*; anti-inflammatory and analgesic agents that reduce swelling and skin irritation and accelerate wound healing

ω-conotoxin MVIIA – Extracted from the cone snail, *Conus magnus*; potent pain-killer

This list reflects an interesting fact about invertebrates that produce pharmacologically active substances: most species are sessile; they are immobile and live all or most of their lives attached to some sort of surface. Several reasons have been suggested to explain why these particular animals produce potent chemicals. One possibility is that they use these chemicals to repel predators, since they are sessile, and thus basically "sitting ducks." Since many of these species are filter feeders, and consequently are exposed to all sorts of parasites and pathogens in the water, they may use powerful chemicals to repel parasites or as antibiotics against disease-causing organisms. Competition for space may explain why some of these invertebrates produce anti-cancer agents: if two species are competing for the same piece of bottom space, it would be helpful to produce a substance that would attack rapidly dividing cells of the competing organism. Since cancer cells often divide more rapidly than normal cells, the same substance might have anti-cancer properties.

The goal of the 2003 Medicines from the Deep Sea Expedition is to discover new resources with pharmaceutical potential in the Gulf of Mexico. To achieve this goal, the expedition will

- collect selected benthic invertebrates from deep-water bottom communities in the Gulf of Mexico (sponges, octocorals, molluscs, annelids, echinoderms, tunicates), identify these organisms, and obtain samples of DNA and RNA from the collected organisms;
- isolate and culture microorganisms that live in association with deep-sea marine invertebrates;
- prepare extracts of benthic invertebrates and associated microorganisms, and test these extracts to identify those that may be useful in treatment of cancer, cardiovascular disease, infections, inflammation, and disorders of the central nervous system;
- isolate chemicals from extracts that show pharmacological potential and determine the structure of these chemicals;
- further study the pharmacological properties of active compounds; and

- develop methods for the sustainable use of biomedically-important marine resources.

The last objective is particularly important, since many potentially useful drugs are present in very small quantities in the animals that produce these drugs. This makes it impossible to obtain useful amounts of the drugs simply by harvesting large numbers of animals from the sea. Some alternatives are chemical synthesis of specific compounds, aquaculture to produce large numbers of productive species, or culture of the cells that produce the drugs. Some techniques for producing specific drugs are based on the cells' own machinery for chemical synthesis: enzymes, guided by information contained in the cells' DNA and RNA.

This activity is designed to acquaint students with bioassays, a test that uses biological organisms to study the action of chemicals or physical changes in the environment.

Learning Procedure

1. Discuss the importance of finding new drugs for the treatment of cardiovascular disease, cancer, inflammatory diseases, and infections. Describe the potential of marine communities as sources for these drugs, and briefly discuss some potentially useful drugs that have been discovered from these communities. Ask students to list some reasons that these kinds of drugs might be found primarily among sessile invertebrates. Briefly introduce the objectives of the 2003 Medicines from the Deep Sea Expedition. Highlight the initial steps in the search for new drugs, and tell students that they will be learning to use a technique for studying the effects of chemicals on living organisms. Explain that a bioassay uses a biological organism to study the effects of chemicals or physical environmental change (such as radiation or heat). When toxicity is being studied, bioassays provide an integrated measure of all changes to which a test organism is suggested, and provide a different type of understanding than would be obtained from direct measurements of specific chemical or physical factors.

   Tell students that they will be using radish seeds as a bioassay organism. Two responses will be investigated: germination and growth rate. Lead a discussion to identify one or more substances (liquids are easiest) whose toxicity is to be tested. Runoff water from a street (usually contaminated with vehicle emissions) or a nearby water body suspected of being polluted are common test subjects. Have students collect the substances to be tested. A sample of 100 ml is adequate for the test.

2. Have students perform bioassays using the following procedure adapted from an article by Joe Rathbun in the spring 1996 issue of the *Volunteer Monitor*:
   a. Soak seeds for 20 minutes in a 10% solution of household bleach in distilled water, then rinse 5 times with distilled water. The solution kills fungi, which could interfere with seed germination.

   b. Place 9-cm filter paper circles into labeled 10-cm plastic petri dishes. Three replicate dishes per sample are recommended.

   c. Pipette enough undiluted sample into the dishes to saturate the filter paper. Use the same volume in all tests. Also prepare 3 control dishes, using distilled water instead of sample.

   d. Place 10 seeds on the paper, spaced evenly. Place the covers over the dishes.

   e. Incubate dishes at room temperature, in the dark, for 5 days. (It is OK to briefly check the dishes during incubation. If the paper seems dry, pipette a few ml of distilled water onto the paper.)

   f. When incubation is complete, students should record the number of seeds that germinated in each plate, and measure (to the nearest mm) the length of the root that has emerged from each germinated seed. If fewer than 80% of the seeds in the control sample germinate, this indicates a problem with the assay (e.g., bad seeds, poor incubation conditions). If this happens, the test should be rerun.

g. For each sample (including the controls), calculate the mean and standard deviation of root lengths. Comparisons can be made by using the Student's t-test. A more approximate method is to compare the mean ±1 standard deviation of each sample to the control. If a sample's mean plus 1 standard deviation is less that the mean of the control minus 1 standard deviation, there's a strong likelihood that the sample is significantly more toxic than the control. Each group should prepare a written report of their results, including a discussion of the outcome.

3. Lead a discussion of students' results. Students should realize that different organisms are not equally sensitive to chemical agents. For example, the concentration of copper in water that would kill algae or a snail is harmless to most fish. When choosing a bioassay organism, investigators need to consider which compounds or organism responses are of most concern. Seed bioassays are very sensitive to herbicides and fairly sensitive to metals. They are less sensitive than fish or invertebrate assays to industrial chemicals like PCBs or solvents. A full evaluation of a sample's biological activity requires performing several different bioassays. Bioassays for drug screening, for example, often include bacteria (to screen for potential antibacterial activity) and specific tissue cultures (to screen for anti-cancer activity).

The BRIDGE Connection

www.vims.edu/bridge/ – Click on "Ocean Science" in the navigation menu to the left, then "Chemistry" for resources on drugs from the sea. Click on "Ecology" then deep sea for resources on deep-sea communities. Click on "Human Activities" then "Technology" then "Biotechnology" for resources on biotechnology.

The "Me" Connection

Have students write a short essay on how bioassays might be of personal benefit.

Connections to Other Subjects

English/Language Arts; Mathematics (Statistics)

Evaluation

Written reports prepared in Step 2 offer opportunity for assessment.

Extensions

Log on to http://oceanexplorer.noaa.gov to keep up to date with the latest discoveries of the 2003 Medicines from the Deep Sea Expedition

Visit http://www.epa.gov/volunteer/fall99/pg01a.html for more examples and ideas for using bioassays.

Resources

http://oceanica.cofc.edu/activities.htm – Project Oceanica website, with a variety of resources on ocean exploration topics

Rathbun, J. 1996. A Simple Bioassay Using Lettuce Seeds. The Volunteer Monitor. Spring, 1996. Available online at http://www.epa.gov/volunteer/spring96/index.html

http://www.reefcheck.org/headlines/june/pdf/marine_pharmacology.pdf – Marine pharmacology

Faulkner, D. J. 2000. Marine pharmacology. Antonie van Leeuwenhoek 77: 135-145. Available online at http://www.reefcheck.org/headlines/june/pdf/marine_pharmacology.pdf.

www.nci.nih.gov – Website of the National Cancer Institute

http://www.woodrow.org/teachers/bi/1993/ – Background and activities from the 1993 Woodrow Wilson Biology Institute on biotechnology

http://spikesworld.spike-jamie.com/science/index.html – Website with lots of background and activities on multiple science topics

National Science Education Standards

Content Standard A: Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Content Standard C: Life Science

- Behavior of organisms

Content Standard F: Science in Personal and Social Perspectives

- Personal and community health
- Natural resources
- Environmental quality

For More Information

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